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Microcontact Printing via a Polymer-Induced Liquid-Precursor (PILP) Process

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ABSTRACT

Our biomimetic approach for patterned crystallization is based on the combination of the Micro-Contact Printing technique and a novel mineralization process, called the Polymer-Induced-Liquid-Precursor (PILP) process, which enables the deposition of mineral films under low-temperature and aqueous-based conditions. We demonstrate that a liquid-phase mineral precursor is deposited onto specific areas templated with self-assembled monolayers of alkanethiolate on gold, and then the patterned calcitic films grow under constrained conditions via transformation of the PILP phase, leading to control over the location and morphology of calcitic films.

INTRODUCTION

The patterning capabilities of inorganic films derived from a biomimetic, bottom-up approach, are of interest in microelectronics and bioelectronics applications that require high performance mechanical, electrical and/or optical properties resulting from controlled nano- and microstructural design. Biomimetic processing techniques are also desirable for biomedical applications that incorporate thermally sensitive components, such as proteins or cells, into devices such as biochips for sensor applications, bioseparations, biocatalysis, and hard-tissue biomaterials. Biological mineralization differs from traditional crystallization in that the crystallization process is mediated with organic materials, leading to a high degree of control over the mineral properties. This occurs through the incorporation of both soluble proteins, which are thought to modulate crystal shape, and an insoluble matrix, which presumably regulates crystal nucleation [1], and enhances the mechanical properties of the bioceramic composite. The performance of synthetic inorganic materials could be significantly advanced by precise control over crystal size, orientation, morphology, and location, as occurs in biominerals; yet rarely can control over all of these properties be accomplished in one synthetic system [2, 3].

We have proposed that a polymer-induced liquid-precursor (PILP) process may play a fundamental role in biomineralization (in both vertebrates and invertebrates) [4,5], and if the mechanisms utilized by biomineralizing systems can be determined, significant advances could occur in the biomimetics field. In the PILP process, micromolar quantities of acidic polymers are added to the crystallizing solution of an inorganic salt (such as calcite), and the charged polymer sequesters the ions and generates liquid-liquid phase separation. The phase boundaries of the minor phase ultimately define the shape of the final crystal products that form upon solidification and densification of the precursor phase, thus producing a variety of non-equilibrium crystal morphologies [5,6]. The elaborate morphologies and composite structures found in biominerals have long been the envy of the materials engineer. We believe the PILP process offers a relatively simple means for mimicking such structures [7].

In addition to crystal morphology, it is the arrangement of the biominerals within the biopolymer matrix that distinguishes bioceramic composites. The design of complex macromolecular structures to serve as templates presents many challenges with respect to molecular recognition at the organic-inorganic interface due to the flexibility of the organic substrate, and the difficulty in producing patterned structures to serve as templates and matrices. Nevertheless, there have been several studies demonstrating the oriented growth of calcium carbonate crystals on self-assembled monolayers (SAMs), as well as amphiphilic and macromolecular surfaces with functionality designed to interact with the incipient nuclei [8-10]. Thin film formation of calcite by template-mediated nucleation on insoluble and relatively rigid surfaces, such as porphyrin monolayers and chitosan, has also been investigated [1, 11, 12]. Our system differs from these in that both morphological and locational control can potentially be achieved due to the shape retention that occurs when patterning a precursor phase, as opposed to controlled surface nucleation, which relies on strict control of the supersaturation and diffusional distances between patterning elements that create ion depletion zones. In the PILP process, an important distinction in terms of morphological control is that a phase segregated precursor phase can conceivably be manipulated, molded and shaped by a compartment since the precursor is in the form of a moderately viscous liquid which takes the shape of its container. In some ways, the PILP phase is analogous to sol-gel precursors used in the low temperature synthesis of ceramics, for which micromolding techniques have already been demonstrated. The difference here, though, is that the mineral precursor is not a chemical precursor, but rather a physical phase created by ion sequestration by the polyanionic additive; and a chemical by-product is not released via a condensation reaction, but rather the hydration waters are driven off (while still in solution) by the thermodynamic instability of this metastable phase.

Template-mediated nucleation of calcite from solution has provided some insight into the molecular recognition affecting the nucleation of the inorganic crystals on organic substrates. However, template-mediated nucleation of calcite crystals formed via transformation of a precursor phase might be expected to behave in an entirely different fashion than crystals nucleated from solution. The template will likely mediate where the precursor phase deposits (influencing location and morphology), and then how it transforms into the crystalline phase (influencing crystal phase, orientation, and polycrystallinity). Therefore, the influence of organic templates on the PILP process is a primary focus of our current work.

EXPERIMENTAL

Patterned Self-Assembled Monolayers (SAMs)

Clean fused silica cover-slips were coated with Au in thickness 500 ~ 1000 Å. (Ti underlayer is used to promote adhesion), using an electron beam evaporator. SAMs were patterned on the Au substrates using the microcontact printing technique [13]. Elastomer stamps were prepared by casting and curing Poly(dimethylsiloxane) (PDMS, SYLGARD 184 (Dow Corning)) against a silicon wafer bearing photoresist patterns formed using conventional photolithographic techniques. The resultant PDMS stamps with relief structures, such as channels and squares, were "inked" with a 1mM solution of surfactant (Mercapto-hexadecanoic acid $SH(CH_2)_{15}COOH$ in Ethanol) and then printed onto the gold surface for 30-60s. The stamps

then were removed, and the printed substrates were washed with ethanol [8]. In the examples shown here, a follow-up dipping of a second surfactant was not utilized.

PILP Crystallization

The crystallization process is as follows: a 12mM $CaCl_2 \cdot 2H_2O$ (Aldrich) solution was prepared with freshly boiled and cooled distilled water. Substrates, Au-coated cover-slips with patterned surfactants, were placed in a petri dish (3.5 cm diameter) containing a filtered 12mM $CaCl_2 \cdot 2H_2O$ solution. Micromolar quantities of dissolved acidic polypeptides (poly-L-aspartic acid, Mw=8600, Sigma) were transferred into the dish by micropipette. The dishes were then covered with stretched parafilm®, punched with 3 needle holes, and placed in a large desiccator. A small vial cap containing crushed ammonium carbonate was also covered with stretched parafilm, punched with 3 needle holes, and placed in the same desiccator. The samples were kept at 4°C (in a refrigerator) or 23°C (room temperature) for 7 days, at which time the cover-slips were removed and gently rinsed with water and ethanol to remove any soluble salts.

The cover-slips were examined by optical microscopy, and then gold coated for scanning electron microscopy on a SEM JEOL JSM 6400 instrument [5].

RESULTS AND DISCUSSION

Microscopic examination of the patterned calcite in polarized light microscopy shows that crystallization without the addition of poly-aspartic acid yields rhombohedral-shaped calcite crystals, as expected. Figure 1A shows the traditional solution crystallization of calcite without a SAM template, and Figure 1B shows a linear array of crystallites patterned using a SAM template prepared with a PDMS stamp containing micro-channels. Figures 1C and 1D show that the addition of micromolar quantities of poly-aspartic acid, which induces the PILP process, cause the deposition of patterned *films* of calcite. It should be noted that some small crystalline aggregates are also present due to the incomplete inhibition of solution crystallization. The addition of magnesium ions as additional nucleation inhibitors helps to eliminate some of these undesirable crystal aggregates, but further optimization of the polymeric additive and crystallizing conditions are still needed. Nevertheless, the different effect produced by the presence of poly-aspartic acid is apparent- films, rather than discrete polycrystals, are deposited on the patterned regions of the SAM template. There may be cooperative interplay between the carboxylate groups of the SAMs and the carboxylate groups on the polypeptide, which could be adsorbed onto the surface. However, we believe that the mechanism requires most of the polymer to exist in solution in order to interact with the ions and generate the metastable phase, which then transforms into the crystalline phase. The deposition of precursor, rather than nucleation of crystals, is now dependent upon on the functional head groups of the SAM template. The crystal nucleation event subsequently occurs via a precursor transformation mechanism, which is also likely modulated by the template. Regarding the deposition stage, it is not certain at this time whether PILP droplets are depositing onto the patterned regions, or if heterogeneous nucleation of an amorphous precursor phase is occurring, or both.

SAMs have found numerous applications in the realm of biomimicry. Controlled growth of crystals with specific orientation can be achieved via the functional groups on the substrate. In

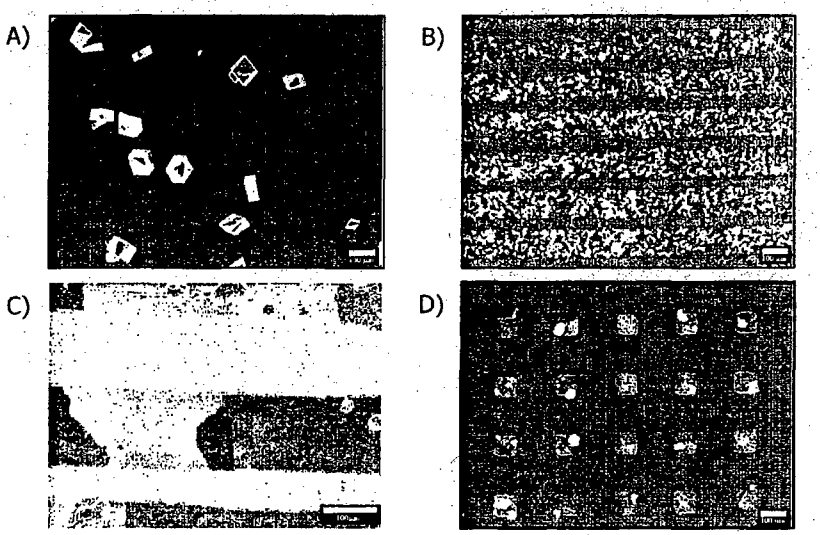

Figure 1. Optical micrographs of calcite crystals produced by slowing raising a 12mM $CaCl_2$ solution in supersaturation with ammonium carbonate vapor. A) Classical rhombohedral shaped calcite crystals are produced in the control reaction, which does not contain a SAM template or soluble polymeric additive. B) Patterned nucleation of calcite crystals produced with 24mM Ca^{2+} deposited onto a SAM template patterned in the form of channels (without soluble polymeric additive). Note- the crystals are smaller here because a higher ion concentration was used, which increased the nucleation density. C) With the addition of 12 µg/ml Poly-L-aspartic acid, the same channel-patterned SAM directed the deposition of patterned mineral films, using otherwise similar precipitation conditions. D) Likewise, square-patterned calcitic films were produced using 12mM Ca^{2+} and 12 µg/ml Poly-L-aspartic acid. The dark objects in C) and D) are crystal aggregate side-products that form from incomplete inhibition of solution crystal precipitates. Scale bars are 100 µm.

fact, Aizenberg has demonstrated patterned crystallization in conjunction with controlled crystallographic orientation. She suggests that the patterned calcite crystallization could be explained through a direct epitaxy nucleation in terms of diffusion-limited nucleation [9, 10]. Although her experiments demonstrate control over the initial nucleation of crystals, the growth stage is not affected by the template, thus crystal morphology is not regulated.

Figure 2 shows scanning electron micrographs of the patterned films, demonstrating both regions of smooth continuous film, as well as bumpy regions, in which the precursor droplets did not fully coalesce. In Figure 2B, bulges can be seen on the film which appear to be "dewetting" of the SAM/mineral film. Although the surface energetics of the precursor phase have not yet been examined, it has been observed that the substrate upon which these films are deposited can have a pronounced affect on the wetting behavior and adhesiveness of the films (for example, the mineral films adhere well to a glass coverslip, but tend to peel off a polystyrene petri dish).

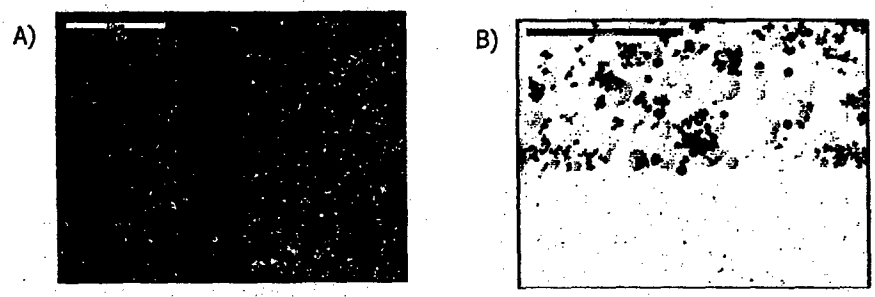

Figure 2. Scanning electron micrograph of patterned mineral films. A) Using 12 mM Ca^{2+} and 12 μg/ml Poly-acrylic acid, the films appear rough due to PILP droplets which have not fully coalesced. Scale bar = 50 μm. B) At higher magnification, it can be seen that the mineral film is relatively smooth, except for some clusters of calcitic droplets. Bulges are also seen in this film, which appear to be associated with "dewetting" of the film from the SAM substrate. Scale bar = 20 μm.

As we expected, the crystalline films were deposited only on the regions that were functionalized. Reasonably well-defined patterned films of calcite are observed, although the fidelity is not optimal. A primary difficulty we have encountered is the formation of crystal aggregate side-products (which appear to be solution grown crystals, rather than PILP formed crystals). Likewise, no control has been exerted over the nucleation of this precursor phase (i.e. transformation via dehydration and crystallization); therefore, the patches are not always single crystalline if more than one nucleation event occurred, and specific crystallographic phase and orientation have not been addressed. It is anticipated, however, that the nucleation of the precursor phase, although different from nucleation from solution, will be amenable to regulation once the process is better understood. Nevertheless, the patterning of this PILP phase can be seen as being distinctly different than the patterning of solution grown crystals. Thus, our approach has taken the microcontact printing technique one step further, making it possible to provide some level of morphological control in patterning thin-film structures of calcium carbonate, by utilizing the synergistic interplay between a SAM template and a soluble acidic polypeptide. It should be noted that two of the crucial tools utilized in biomineralization are insoluble templates and soluble acidic proteins. We have used acidic polypeptides, such as poly-aspartic acid, to mimic the soluble polyanions found in biominerals, and the patterned SAMs serve the role of insoluble matrix found in biological hard tissues. One can imagine that by controlling the location of the organic template, sequential depositions of PILP phase could enable the fabrication of hierarchical composite structures, as occurs in biomineralized tissues. A more detailed discussion of the potential relevance of the PILP process to biomineralization is discussed in more detail in our other reports [4, 7].

CONCLUSIONS

Micro-scale patterning of calcite films via a polymer-induced liquid-precursor (PILP) process has been achieved using soluble acidic polypeptides, such as poly-L-aspartic acid (or poly-acrylic acid), in conjunction with micro-contact printing techniques. This demonstrates a

new strategy for the controlled deposition and patterning of inorganic thin films under benign processing conditions. Our future efforts will be directed at better controlling the precursor formation to eliminate the problem of crystal aggregates and produce patterned films with improved smoothness and higher resolution and reproducibility.

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REFERENCES

1. L.A. Gower and D.A. Tirrell, *J. Cryst. Growth* **191**, 153-160 (1998).
2. S. Mann, *J. Mater. Chem.* 5, 935-946 (1995).
3. S. Mann, D.D. Archibald, J.M. Didymus, T. Douglas, B.R. Heywood, F.C. Meldrum, and N.J. Reeves, *Science* **261**, 1286-1292 (1993).
4. M. J. Olszta, E.P.Douglas, L.B. Gower, *Calcified Tissue International* (submitted).
5. L.B. Gower and D.J. Odom, *J. Cryst. Growth* **210**, 719-734 (2000).
6. L. Gower, "The influence of polyaspartate additive on the growth and morphology of calcium carbonate crystals", Dissertation, University of Massachusetts- Amherst (1997).
7. M. J. Olszta, E.P. Douglas, L.B. Gower, *Connective Tissue Research* (in Press).
8. J. Aizenberg, *J. Cryst. Growth* **211**, 143-148 (2000).
9. J. Aizenberg, A.J. Black, and G.H. Whitesides, *J. Am. Chem. Soc.* **121**, 4500-4509 (1999).
10. J. Aizenberg, A.J. Black, and G.M. Whitesides, *Nature* **398**, 495-498 (1999).
11. N. Hosoda and T. Kato, *Chem. Mat.* **13**, 688-693 (2001).
12. G.F. Xu, N. Yao, I.A. Aksay, and J.T. Groves, *J. Am. Chem. Soc.* **120**, 11977-11985 (1998).
13. Y.N. Xia and G.M. Whitesides, *Annu. Rev. Mater. Sci.* **28**, 153-184 (1998).